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ORIGINAL ARTICLE

The effect of salinity on jungle perch *Kuhlia rupestris* egg buoyancy and larval hatch rates

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Abstract

This paper reports on the first ever observations of jungle perch *Kuhlia rupestris* egg buoyancy and hatch rates over a range of salinities and represents important information for the husbandry of this species. Dead or unfertilized jungle perch eggs tend to sink in static seawater (>34,000 mg/L), whereas fertilized eggs tend to float. However, at salinities below 32,000 mg/L most fertilized eggs also sink, making it difficult to separate them from unfertilized eggs. Separating fertilized from unfertilized and dead eggs is important for maintaining water quality in aquaculture hatch tanks. Hatch rates of fertilized jungle perch eggs were significantly higher ($p < 0.05$) at salinities at or above 32,000 mg/L than at lower salinities. The highest mean buoyancy and hatch rates were recorded at 36,000 mg/L. This suggests that jungle perch are fully marine, rather than estuarine spawners. It is recommended that fertilized jungle perch eggs be held at salinities of 32,000 mg/L or higher to maximize hatch rates and to facilitate removal of dead egg material from hatch tanks.

KEYWORDS
egg buoyancy, hatch rates, hatchery, jungle perch, *Kuhlia rupestris*, rock flagtail, salinity

1 | INTRODUCTION

Jungle perch (also known as rock flagtail) *Kuhlia rupestris* have a wide Indo-Pacific distribution, stretching from east Africa and Madagascar to India and parts of south-east Asia, including Indonesia, Malaysia and the Philippines. In the Pacific, their range extends north to Taiwan and southern Japan, south to northern New South Wales in Australia and east to Fiji and Samoa (Gelineau et al., 2020; Hutchison et al., 2002).

In Queensland, Australia, jungle perch are a popular recreational fishing target (Allen et al., 2002) and have recently been declared a candidate species by the Queensland Department of Agriculture and Fisheries for stocking into coastal impoundments for recreational fisheries enhancement programmes (Fisheries Queensland, 2020). There is increasing interest in culturing this species in Queensland for conservation stocking, recreational fisheries enhancement and grow-out for aquaculture. In the Philippines and Vanuatu, fisheries agencies have expressed an interest to progress hatchery production of this species for restocking for artisanal fisheries and for aquaculture (Madel L. Canceran, Research specialist, NFRDI-Fisheries Biotechnology Center, Philippines; June Brian Molitaviti, Manager, Research and Aquaculture Division of the Vanuatu Fisheries Department. pers. com).

Jungle perch are a catadromous species of fish, with juveniles, sub-adults and adults occupying freshwater habitats. Based on evidence from otolith microchemistry from across the Indo-Pacific, spawning takes place in marine environments and marine larval duration ranges between 27 and 58 days before the fry enter freshwater

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(Feutry et al., 2012). Jungle perch from Fraser Island, Queensland, were found to have a narrower marine larval duration range of 35–45 days (Hamer et al., 2015).

Information on optimal spawning salinities is variable. Henderson (2010) found the highest sperm activity levels of jungle perch sourced from Wyuna Creek on Fraser Island occurred at a salinity of 36,000 mg/L, with the duration of sperm activity lasting for 8 min at both 34,000 mg/L and 36,000 mg/L. At 30,000 mg/L sperm activity was too low for effective fertilization to occur, indicating full salinity seawater was necessary for effective spawning. In contrast, Hogan and Nicholson (1987) reported the optimal salinity for sperm motility of jungle perch from Saltwater Creek in north Queensland was 25,000 mg/L and suggested that jungle perch most likely spawned at salinities between 25,000 mg/L and 32,000 mg/L.

During pilot work the authors observed that fertilized jungle perch eggs were buoyant in static seawater (>34,000 mg/L), whereas unfertilized and dead eggs tended to sink. This trait is useful to separate fertilized from unfertilized eggs. However, the range of salinities over which jungle perch eggs remained buoyant was unknown, as was the effect of salinity on hatch rates. In this paper, the effect of salinity on the buoyancy of fertilized eggs and on the hatch rate of jungle perch larvae was investigated. The objective of this work was to assist with improving hatchery management of fertilized jungle perch eggs.

2 | METHODS

All work with live fish was approved by the Queensland Department of Agriculture and Fisheries Community Access Animal Ethics Committee, under permit number CA 2011/05/509. Fish were also held under a Queensland General Fisheries Permit (GFP 147714).

2.1 | Spawning induction

Broodstock were originally sourced from two main regions: the Mackay-Townsville region (central to northern Queensland) and Fraser Island (south-eastern Queensland). Shortly after collection from the wild, all broodstock were PIT tagged and the sex of fish was determined by cannulation of oocytes with a 2-mm external diameter plastic tube. If the cannula could not be inserted into the urogenital opening, the fish was usually male, or rarely an immature female. Males were confirmed by expression of milt.

Jungle perch broodstock held in 7000 L low salinity (3000 mg/L) tanks were induced to spawn by injection of Ovaprim ®(Syndel) injectable solution (salmon GnRH 20 μg.ml^{-1} with domperidone 10 mg.ml^{-1}) at a dosage of 1 ml.kg^{-1} bodyweight for both sexes. The effective dosage rate for this species was determined from earlier unpublished pilot work by the authors of this paper. The standard dose used for most fish species is 0.5 ml.kg^{-1} (Kucharczyk et al., 2020, 2021). Females were considered suitable for spawning induction if at least 50% of cannulated oocytes were ≥380 μm in diameter. This species is a multi-batch spawner, so it is normal for oocytes to be at a range of developmental stages. Males were considered suitable for spawning induction if a show of milt could be expressed by gentle abdominal pressure. For each spawning event, one female jungle perch was stocked in a 7000-L spawning tank with three males. The size of females used ranged from 844 g to 2987 g and the size of males ranged from 231 g to 975 g. Female jungle perch reach a larger maximum size than males, but the species is not protandrous.

Four spawning tanks were used for each spawning event. Spawning tanks were initially of low salinity (3000 mg/L), identical to the broodstock tanks. After the induced fish were stocked into the spawning tanks, the water level was lowered to 25% capacity and the tank switched to a marine water recirculating system (supplemented with a trickle of UV-C treated filtered seawater) for filling. Salinity levels in the spawning tank rose to full seawater levels (at least 34,000 mg/L) after 12 h. Water in the spawning tanks was maintained at 28°C using a combination of room air conditioning, 3000 W immersion heaters and a heat exchanger through which 10% of recirculated seawater was diverted. Water was circulated through the spawning tanks at a rate of approximately 50 L.min^{-1} or 100% exchange in just over 2 h. Tanks were well aerated with air pumped through two centrally located air-stones. Dissolved oxygen levels recorded in the spawning tank system ranged from 4.87 mg.L^{-1} to 6.38 mg.L^{-1} and the pH ranged from 8.14 to 8.68. Spawning occurred 48–60 h after induction, with a median induction period of 54 h 6 min.

2.2 | Egg collection and separation

Eggs for the salinity experiments were collected from the first good quality spawn among the four spawning tanks. The remaining spawns and eggs were used for other purposes.

Each spawning tank had a 90-mm overflow pipe that ran into a 300-μm mesh egg collecting basket set in a 200-L tub. Overflow from the tub was returned to the recirculating system via an outlet pipe. From 48 h after induction, egg baskets were checked at 15-min intervals. If eggs were detected in a basket they were left to accumulate for an hour before being collected. Eggs were transferred from the basket to a 20-L bucket of seawater. The contents of the bucket were then gently swirled in a circular motion and allowed to settle. Dead and unfertilized eggs settled in the centre of the bottom of the bucket. These were then siphoned out through a 5-mm tube for disposal. Fertilized eggs were positively buoyant and rafted together on the surface. A sub-sample of at least 50 rafting eggs was skimmed off the surface with a 1-L beaker. These eggs were then pipetted to a Petri dish and examined under a dissecting microscope at 20× magnification to confirm all the rafting eggs were fertilized.

Once fertilization was confirmed, the remaining rafting eggs in the bucket were mixed by a gentle stirring action to obtain a homogenous distribution, then six 10-ml subsamples were collected from the bucket by pipette. Each subsample was transferred to a 120-μm mesh sieve, and the number of eggs in each sub-sample was counted with the aid of a dissecting microscope. Once the density of eggs in the bucket was known, sub-samples of approximately 50 eggs were collected volumetrically for use in buoyancy and hatching experiments.

TABLE 1 Statistical significance of spawning event, salinity and spawning event.salinity on buoyancy rate for jungle perch *Kuhlia rupestris* eggs over the salinity range 28,000 mg/L–32,000 mg/L, as analysed by GLM of binomial proportions with logit link function

Source of variation	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. chi *p*.
Spawn	2	37.7766	18.8883	18.89	<0.001
Salinity	4	334.0522	83.5130	83.51	<0.001
Spawn.salinity	8	31.0551	3.8819	3.88	<0.001
Residual	30	17.8089	0.5936		
Total	44	420.6928	9.5612		

Note: Approximate chi probabilities are shown.

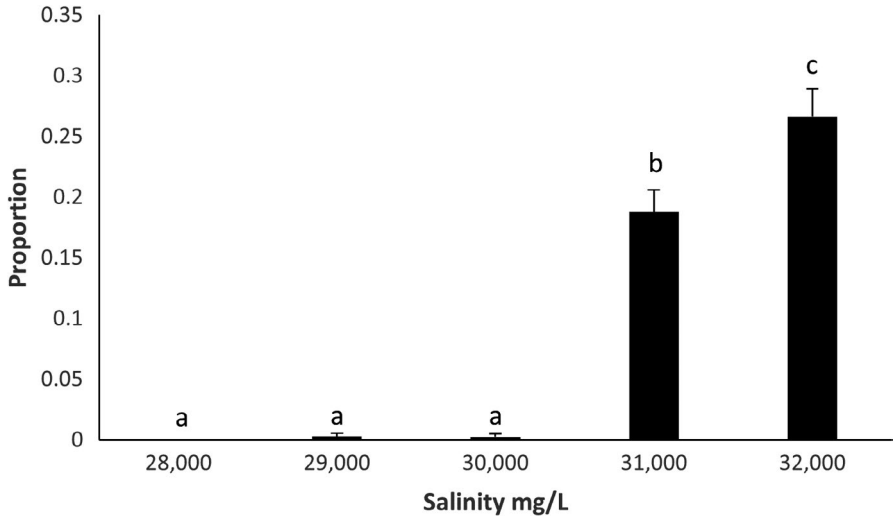

FIGURE 1 Mean proportion (+SEM) of buoyant jungle perch *Kuhlia rupestris* eggs at salinities ranging from 28,000 mg/L to 32,000 mg/L 2 h after fertilization. Categories not sharing the same letter are significantly different ($p \leq 0.05$)

2.3 | Buoyancy and hatch rates

Fertilized eggs collected from spawns in March, April and May 2013 were tested for buoyancy at salinities from 28,000 mg/L to 32,000 mg/L, at 1000 mg/L intervals. Spawns from March and May were from Mackay-Townsville region broodstock, and the spawn from April was from Fraser Island broodstock. Hatch rates were also examined for the April and May 2013 spawns across the same range of salinities. Batches of approximately 50 eggs were transferred volumetrically into 1-L beakers containing each of the test concentrations of seawater. Test concentrations were made up by diluting UV-C-treated 1 μm filtered seawater (sourced via an intake pipe from the ocean adjacent to the Bribie Island Research Centre) with de-ionized water. To achieve salinities higher than that of the incoming seawater, sea salt was added to the beakers. Salinity levels of incoming seawater and test solutions were validated using an Iwaki refractometer (Iwaki, Japan). Three replicate beakers were used for each concentration. Beakers were maintained at a temperature of 28°C in a controlled temperature room.

Two hours after transfer into the beakers, the number of eggs rafting on the surface of each beaker was counted. Jungle perch larval hatching at 28°C was fully completed just under 16 h after fertilization. Approximately 18 h after fertilization, the contents of each beaker were strained through a 200-μm sieve. The number of hatched larvae and unhatched eggs on the sieve were then counted under a dissecting microscope.

In February 2014, the buoyancy and hatch rate of eggs were tested over a wider range of salinities (28,000–36,000 mg/L) at 2000 mg/L intervals. Eggs from this spawn were sourced from Mackay-Townsville region broodstock. As per the previous experiments, three replicate 1-L beakers were used for each salinity level. Methods used were the same as for the previous experiments except buoyancy was estimated 1 h after stocking eggs into the beakers, rather than after 2 h.

2.4 | Statistical analyses

Statistical analyses were completed using GenStat®2021 edition software (VSN international limited). Hatch and buoyancy counts from the 2013 and 2014 spawns were analysed using a generalized linear model (GLM) (McCullagh & Nelder, 1989) with the binomial distribution and logit link. Adjusted mean proportions and their standard errors were estimated, and protected LSD testing was conducted. Salinity and spawn event and their interactions were treated as factors in the model for the 2013 data, while salinity was the only factor considered for the 2014 data as there was only a single spawn event analysed.

3 | RESULTS

The GLM of binomial proportions for egg buoyancy for salinities between 28,000 mg/L and 32,000 mg/L showed that salinity ($p < 0.001$), spawn event ($p < 0.001$) and the interaction between spawn event and salinity ($p < 0.001$) all had significant effects (Table 1). The interaction

between salinity and spawning event indicated that different batches of eggs had different buoyancies, and although increasing salinity was still related to increased buoyancy across all batches, the rate of change differed between batches. Overall, the proportion of buoyant eggs at 28,000–32,000 mg/L were low, not exceeding 30%. Between 28,000 and 30,000 mg/L most eggs were not buoyant, with no buoyant eggs recorded at 28,000 mg/L. Buoyancy rates at these salinities were significantly lower ($p < 0.05$) than at 31,000 mg/L and 32,000 mg/L. Buoyancy rates were also significantly higher ($p < 0.05$) at 32,000 mg/L than at 31,000 mg/L (Figure 1).

The GLM of binomial proportions for hatch rates at salinities 28,000–32,000 mg/L, showed that spawn event ($p < 0.001$) and salinity ($p = 0.023$) had significant effects (Table 2), but there was no significant interaction ($p = 0.388$) between spawn event and salinity for hatch rates. One spawn event had better hatch rates than the other event, but the hatch rates of both batches responded similarly to changes in salinity. Mean hatch rates between 28,000 and 32,000 mg/L were relatively low (not exceeding 35%). The mean proportion of hatched eggs at 28,000 mg/L (19.9%) was significantly lower ($p < 0.05$) than the mean hatched proportion at salinities of 29,000 31,000 and 32,000 mg/L, which ranged between 26.5% and 32.3%. (Figure 2).

The GLM of binomial proportions for buoyancy at salinities 28,000–36,000 mg/L was significant ($p < 0.001$, Table 3). The highest mean buoyancy rates (>70%) were recorded at 36,000 mg/L and the lowest mean buoyancy rates (0%) were recorded at both 28,000 and 30,000 mg/L. Buoyancy rates were significantly higher ($p < 0.05$) at 36,000 mg/L than at any other tested salinity and buoyancy at 32,000 and 34,000 mg/L was significantly higher ($p < 0.05$) than at 28,000 and 30,000 mg/L (Figure 3).

Hatch rates between 28,000 and 36,000 mg/L were significantly affected by salinity ($p = 0.020$, Table 4). The highest mean hatch rate was recorded at 36,000 mg/L (>70%) and the lowest mean hatch rate was observed at 28,000 mg/L (20%). Hatch rates at 34,000 and 36,000 mg/L were significantly higher ($p < 0.05$) than at 28,000 and 30,000 mg/L, and hatch rates at 32,000 mg/L were significantly higher ($p < 0.05$) than at 28,000 mg/L (Figure 3).

4 | DISCUSSION

Only buoyant eggs were collected from full seawater for the experiments described in this study, therefore, all jungle perch eggs were initially buoyant when placed in the beakers at salinities of 34,000 mg/L or higher. The fact that some eggs sank after 1 or 2 h at these higher salinities is probably related to the death of a proportion of those eggs, and this is reflected by the hatch rate data. Only eggs rafting on the surface after 1 or 2 h were classified as buoyant in this series of experiments, but not all non-buoyant eggs sank to the bottom of the beakers. A small proportion of eggs remained neutrally buoyant and visible in the water column.

Spawning event and the interaction between spawning event and salinity were both significant factors at 28,000–32,000 mg/L. It is, therefore, highly likely that egg quality had a role in the buoyancy and hatch rates observed at different salinities. However, the general trends remained consistent between batches. Higher hatch rates and buoyancy rates occurred at the higher salinity levels tested, while the poorest outcomes occurred at 28,000 mg/L.

Baras et al. (2018) state that buoyancy in marine fish eggs is mainly achieved through hydration and not from oil globules in the eggs. About 80% of marine species have eggs with oil globules, and these globules average 2% total egg volume (Baras et al., 2018). Jungle perch oil globule volume is approximately 2.1% of total egg volume (Queensland Department of Agriculture and Fisheries, unpublished data), which is close to the mean value for marine fish. In freshwater fish, those species with pelagic eggs achieve buoyancy through mechanisms other than hydration, such as large oil globules which average 40% of the total egg volume (Baras et al., 2018). In freshwater species without oil globules, buoyancy is thought to be achieved by an increase in the size of the perivitelline space and buoyancy is further assisted by currents in flowing freshwaters (Chen et al., 2021).

Variations in salinity with depth determine the vertical distribution of marine and estuarine fish pelagic eggs, according to the specific gravity of the egg (Sundby & Kristiansen, 2015). Fish species may be adapted to spawn in critical layers that provide an advantage to the development of the egg and the hatched larvae (Sundby & Kristiansen, 2015). In estuarine waters with a salt wedge or halocline present, pelagic eggs may be found on or below the halocline where the salinity is sufficient for buoyancy (Karaseva et al., 2020; Stenevik et al., 2008; Williams et al., 2020). In estuaries, salinities have greater variability, and it is more likely estuary spawned jungle perch eggs would encounter sub-optimal conditions for hatch and buoyancy. Even within the salt wedge, salinities are likely to be too low (<32,000 mg/L) to maintain buoyancy of jungle perch eggs. High salinities for egg incubation appear to be important for jungle perch and high salinities (≥32,000 mg/L) should be maintained in incubation tanks when culturing this species.

TABLE 2 Statistical significance of spawning event, salinity and spawning event.salinity on the proportion of hatched jungle perch *Kuhlia rupestris* eggs over the salinity range 28,000 mg/L–32,000 mg/L, as analysed by GLM of binomial proportions with a logit link function

Source of variation	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. chi *p.*
Spawn	1	243.8537	243.8537	243.85	<0.001
Salinity	4	11.3912	2.8478	2.85	0.023
Spawn.salinity	4	4.1383	1.0346	1.03	0.388
Residual	20	17.5371	0.8769		
Total	29	276.9204			

Note: Approximate chi probabilities are shown.

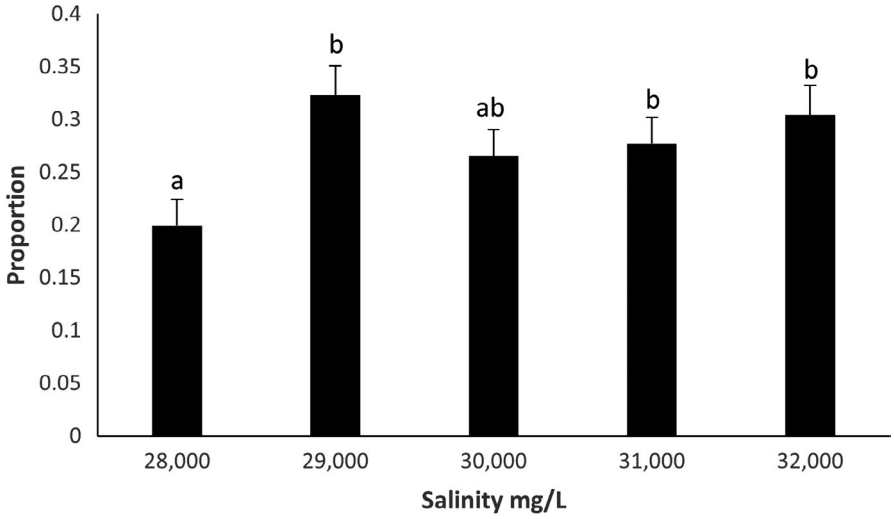

FIGURE 2 Mean proportion (+SEM) of hatched jungle perch *Kuhlia rupestris* eggs at salinities ranging from 28,000 mg/L to 32,000 mg/L. Categories not sharing the same letter are significantly different ($p \leq 0.05$)

TABLE 3 Summary of regression analysis of binomial proportions, with logit link function, for jungle perch *Kuhlia rupestris* egg buoyancy over the salinity range 28,000 mg/L–36,000 mg/L, with approximate F probability

Source of variation	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. F *p.*
Regression	4	345.94	86.485	63.64	<0.001
Residual	10	13.59	1.359		
Total	14	359.53	25.681		

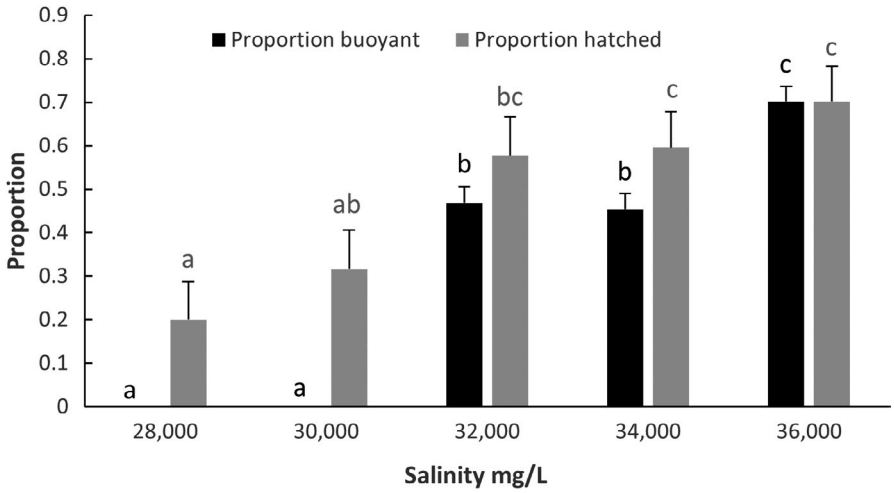

FIGURE 3 The effect of salinity (28,000 mg/L–36,000 mg/L) on egg buoyancy (1 h after fertilization) and larval hatch rates for jungle perch *Kuhlia rupestris*. Categories not sharing the same letter are significantly different ($p \leq 0.05$). Grey letters indicate differences for hatch rates and black letters differences for buoyancy. Bars show mean values + SEM

TABLE 4 Summary of regression analysis of binomial proportions, with logit link function, for jungle perch *Kuhlia rupestris* egg hatching rates over the salinity range 28,000 mg/L–36,000 mg/L, with approximate F probability

Source of variation	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. F *p.*
Regression	4	109.08	27.270	4.81	<0.020
Residual	10	56.74	5.674		
Total	14	165.83	11.845		

In some species of fish, the buoyancy of eggs can be increased by spawning the fish at lower salinities because the fish increase the hydration of their eggs (May, 1974; Petereit et al., 2009). However, this was not feasible for the stocks of jungle perch used in this trial. It was not possible to obtain fertilized eggs from any of these fish at salinities of 30,000 mg/L or less, which confirms the sperm motility observations of Henderson (2010). Hogan and Nicholson (1987) concluded a salinity of 25,000 mg/L was optimal for sperm motility of jungle perch sourced from Saltwater Creek in far north Queensland, but spawning of jungle perch from that region has not yet been trialled.

There can be phenotypical variation in buoyancy of eggs between fish populations of the same species. For example, Atlantic cod *Gadus morhua* from the North Atlantic Basin produce eggs that

are neutrally buoyant at 29,500–33,000 mg/L, whereas populations adapted to the less saline waters of the Baltic Sea produce eggs that are neutrally buoyant at 12,300–18,300 mg/L (Jung et al., 2012). Similarly, there is variation in the buoyancy of Atlantic cod eggs from the less saline Norwegian fjords and the Atlantic Ocean (Stenevik et al., 2008). Whether egg buoyancy of far north Queensland jungle perch populations differs from that of populations further south is yet to be tested, but parallels with optimal spawning salinities could be plausible. It would be useful to know if there is geographical variation in the relationship between salinity and the buoyancy and hatch rates of jungle perch eggs because this could influence hatchery management in different regions.

Broodstock sourced for the work reported in this paper were from the Mackay-Townsville region and Fraser Island. Although both regions experience wet summers, the average wet season rainfall in these regions is much lower than in the wet tropics, where Saltwater Creek is located. Annual average rainfall in Mossman, near Saltwater Creek is 2391 mm, whereas at Townsville it is 1136 mm, at Mackay 1542 mm and at Eurong on Fraser Island 1564 mm (Australian Bureau of Meteorology data). High rainfall and run-off can affect salinities in adjacent marine areas. Thus, it is plausible that during the jungle perch spawning season, mean marine salinities near Saltwater Creek may be lower than those around Townsville, Mackay and Fraser Island.

Like jungle perch, various other marine species have an optimal incubation range above 30,000 mg/L. For example, the optimal salinity range for incubating embryos of devil stringer *Inimicus japonicus* is between 30,500 and 37,300 mg/L (Gong et al., 2018). Hatch rates of Nassau grouper were best between 32,000 and 36,000 mg/L (Ellis et al., 1997) and between 34,000 and 37,000 mg/L for black porgy *Acanthopagrus schlegelii* (Huang et al., 2000). In contrast, Van Der Wal (1985) observed that the catadromous estuarine spawner Australian bass *Macquaria novemaculeata*, had high hatch rates and survival over a wider range of salinities (25,000–35,000 mg/L). Some hatch was still present at salinities as low as 5000 mg/L but with no larval survival, and only limited larval survival at 10,000 mg/L. The euryhaline silver moony *Monodactylus argenteus*, which inhabits brackish water, has optimal hatching rates between 25,000 mg/L and 40,000 mg/L and no hatch at salinities of 5000 mg/L or less (Thomas et al., 2021). Full buoyancy of silver moony eggs was observed at salinities above 30,000 mg/L and with no buoyancy observed at salinities <20,000 mg/L. Neutrally buoyant eggs were observed between 25,000 mg/L and 30,000 mg/L (Thomas et al., 2021). Tawny puffer fish *Takifugu flavidus* also spawn in estuarine areas. Embryo hatching rates were above 70% in this species at salinities ranging from 5000 mg/L to 45,000 mg/L, with the highest hatching rates occurring between 10,000 mg/L and 20,000 mg/L (Zhang et al., 2010).

In general, freshwater spawners, like marine spawners, tend to have narrower preferred salinity ranges than estuarine spawners for egg incubation. For example, the optimal salinity range is 0–3000 mg/L for sichel *Pelecus cultratus* (Kujawa et al., 2017), 0–3000 mg/L for silver perch *Bidyanus bidyanus* (Guo et al., 1993) and 100–5000 mg/L for the Australian grayling *Prototroctes maraena* (Bacher & O'Brien, 1989).

The narrower salinity range for hatching of jungle perch eggs from Mackay-Townsville and Fraser Island broodstock, compared with that of Australian bass, silver moony and tawny puffer fish, further supports the supposition that jungle perch are marine, rather than estuarine spawners.

While hatch rates in static beakers may not exactly replicate what happens in aerated hatch tanks, we believe that the general relationship between hatch rate and salinity levels will still apply. Although some hatch of jungle perch larvae can be achieved at salinities below 32,000 mg/L, eggs that sink to the bottom of tanks are more likely to be susceptible to bacterial infection. Even though these eggs may be kept in suspension by currents in aerated hatch tanks, the inability to separate live eggs from dead eggs at these lower salinities could negatively impact on water quality and, therefore, hatch rates and post-hatch larval survival. Therefore, it is recommended to incubate jungle perch eggs in hatch tanks with salinities in the range 32,000–36,000 mg/L to optimize production.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

MH helped secure funding for this work, was involved in experimental design, running of experiments, data analyses and writing of the paper. AN was involved in experimental design, running of the experiments and writing of the paper, DN was involved in experimental design, running of the experiments and writing of the paper.

DATA AVAILABILITY STATEMENT

Data can be made available on request to the corresponding author.

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